



TITLE:

Studies on the Metabolic Products of *Pseudomonas Aeruginosa* : On the Production of Antibiotic Substances

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CITATION:

Katagiri, Hideo ...[et al]. Studies on the Metabolic Products of *Pseudomonas Aeruginosa* : On the Production of Antibiotic Substances. 京都大学化学研究所報告 1951, 25: 71-72

ISSUE DATE:

1951-09-10

URL:

<http://hdl.handle.net/2433/74266>

RIGHT:

* The numerals are represented the amount of H_3BO_3 mg per Kg of dry matter

1) Boodler coacta (Dickie) Murray et de Toni, 2) Monostroma nitidum Wittrock,
3) Padina arborescens Holmes, 4) Gummy matter from Brasenia Schr. Gmel 5) Coagulated tannin from Kaki-fruits.

As seen in the table polysaccharides occur in natural state combining tightly with boron, probably as borate ester at the polyol radical of polysaccharides.

26. Studies on the Metabolic Products of *Pseudomonas Aeruginosa* On the Production of Antibiotic Substances

Hideo Katagiri, Toyozo Shibutani and Mamoru Kurachi

(Katagiri Laboratory)

With newly isolated strains of *Pseudomonas aeruginosa*, experiments were carried out to find satisfiable conditions on the production of pyocyanine and other antibiotic substances.

It was observed that the formation of antibiotics, especially pyocyanine, was remarkably affected by various cultural conditions; kinds of nutrients or pH of the medium.

Any pigment formation and antibacterial activity were never revealed, when glucose solution (higher than 2%) was employed for glycerol which is regarded generally as a suitable source of carbon. However, the same antibacterial activity and pyocyanine formation were revealed in the medium containing 3% glucose as were observed by glycerol medium, when the solution was kept alkaline (pH 7.8-8.8).

Among various kinds of cultural solutions, the following medium was chosen for a suitable solution for the formation of pyocyanine: 3 g glycerol, 1 g peptone, 0.1 g asparagine or glutamic acid, 0.1 g NH_4NO_3 , 0.025 g K_2HPO_4 , 0.025 g $MgSO_4 \cdot 7H_2O$ and 0.0005 g $Fe_2(SO_4)_3 \cdot 10H_2O$ were dissolved in 100cc of distilled H_2O , and then adjusted pH to 7.4 (incubation temperature was 37°C).

The acid hydrolyzate of peptone was useful for source of nitrogen, but the mixture of nitrogenous matters mentioned above was found to be indispensable for the formation of pyocyanine, since no pyocyanine formation was observed with one of these nitrogen sources.

For mineral matters, K, Fe, phosphate, sulphate and especially Mg, without which pyocyanine formation never observed, were found to be essential.

The other antibiotic substances than pyocyanine were suggested to be produced by the bacteria, since no pigment formation was observed and yet noticeable antibacterial power was pointed out with some cultural solution containing different amounts of the nutrients mentioned above.

It will be worth to note that only a trace of pigment was produced in organic media such as Bouillon, yeast water etc., although abundant growth of the bacteria was observed in these media, and that growth of bacteria and the formation of pyocyanine were accelerated by the culture under aeration.

27. Study on Aromatic Stibonic Acid. (V)

Preparation of the Arylstibonic Acid Derivatives

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It has been reported that the phenylstibonic acid is obtained by the decomposition of the benzenediazonium tetrachloroantimonite complex in various organic solvents.* The influences of the substitution group on the decomposition reaction of the complex salts were examined.

Adding a concentrated hydrochloric acid solution of antimony trichloride to the p-methylbenzenediazonium chloride, the p-methyl derivative of the complex $[\text{CH}_3\text{C}_6\text{H}_4\text{N}_2] \text{SbCl}_4$ was obtained as a yellow fine crystal (yield 92%). In alkaline solution, p-methyl derivative decomposed at 56°C, to p-chlorotoluene (49%), toluene (17.4%) and small amount of biphenyl derivatives. In the presence of Cu_2Cl_2 , however, the chief product was p-tolylstibonic acid (87%).

p-Methoxybenzenediazonium tetrachloroantimonite complex was prepared from p-anisidine and antimony trichloride, by a similar method and obtained a deep yellow fine crystal, $[\text{CH}_3\text{OC}_6\text{H}_4\text{N}_2] \text{SbCl}_4$ (yield 70%). The methoxy derivatives decomposed in acetone solution at 56°C, to p-chloroanisole (54%) and anisole (17%), but in the presence of Cu_2Cl_2 , p-anisylstibonic acid was separated as the main product (52%). p-Nitroaniline was converted to the corresponding complex compound $[\text{NO}_2\text{C}_6\text{H}_4\text{N}_2] \text{SbCl}_4$, as an orange precipitate with the yield of 88%. The decomposition of the p-nitro derivative in alkaline solution occurred at -5~0°C and p-nitrophenylstibonic acid (70%) and resinous matter were liberated. The nitro complex in acetone solution decomposed at 15°C, to nitrobenzene (12%), p-chloro-nitrobenzene (52%) and with comparatively large amount of resinous matter and in the presence of Cu_2Cl_2 , the chief product was p-nitrophenylstibonic acid with the yield of 55%. From the above results it is conceivable that p-methyl group favours to the formation of arylstibonic acid, while p-methoxy and p-nitro group are not favourable as they accompany with the resinification.

* Tomono: This Bulletin, 21 41, 22 49.